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Developmental Expression of the Type I Diabetes Related Antigen Sulfatide and Sulfated Lactosylceramide in Mammalian Pancreas

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Abstract Previous studies have shown that sulfatide is present and functionally involved in beta cells, and that anti-sulfatide antibodies (ASA) exist during development of type I diabetes mellitus. To further explore the possible role of sulfatide in type I diabetes, developmental expression was examined in human pancreas and in pancreas of the type I diabetes models BB rat and NOD mouse compared to Lewis rat and BALB/c mouse, respectively. Sulfatide was not only expressed in adult pancreas, but also in human fetal and rodent neonatal pancreas, i.e., during the growing period of the immunological self. Sulfatide had a different expression pattern in human beings and rodents, concerning both the amounts of sulfatide and expression during development. There was no change in the sulfatide fatty acid isoform expression during development. The pancreatic expression of another sulfated glycosphingolipid, sulfated lactosylceramide, indicated that this molecule is a potential fetal/neonatal marker, which was further expressed in the type I diabetic models. In conclusion, these findings give further support to the possibility that sulfatide is a relevant autoantigen in type I diabetes and that sulfated lactosylceramide might function as a potential risk factor for disease development, at least in the animal models. J. Cell. Biochem. 89: 301–310, 2003.

Key words: sulfatide; sulfated lactosylceramide; glycosphingolipid; type I diabetes mellitus; animal model; fetal; neonatal

Sulfatide is known as a neuronal epitope present in large amounts in the central and peripheral nervous system mainly located to the myelin [Vos et al., 1994], where it has been found to be of importance for the maintenance of the structure and function of the myelin sheet [Coetzee et al., 1996]. The association of type I (insulin dependent) diabetes mellitus with the Stiff-man syndrome [Baekkeskov et al., 1990], and possibly with Guillian-Barré syndrome [Rabinowe, 1990], and the findings of paresis and diabetes in the encephalomyocarditis virus mouse model [Buschard, 1985], suggest the occurrence of a common antigen between endocrine pancreas and nerve tissue. Sulfatide has been investigated as one plausible candidate antigen. Sulfatide is present in the plasma membrane and in secretory granules of pancreatic beta cells [Buschard et al., 1993b] and anti-sulfatide antibodies (ASA) are found in patients with newly diagnosed type I diabetes [Buschard et al., 1993a]. ASA was also found in

Abbreviations used: C/M/W, chloroform/methanol/water; ESI-MS, electrospray ionization-mass spectrometry; TLC-ELISA, thin-layer chromatography-enzyme linked immunosorbant assay; ASA, anti-sulfatide antibodies; CST, cerebroside sulfotransferase.

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the type I diabetes model, the BB rat [Buschard et al., 1993b]. Regarding other tissues, sulfatide has been found to be located in distal tubulus of kidney, and in pericytes in the choroid layer of the eye [Buschard et al., 1994]. The finding of sulfatide in both the islets of Langerhans and in organs affected later in the diabetic syndrome, supports a possible pathogenic connection.

Sulfatide is synthesized from sphingosine and a fatty acid to form ceramide, to which galactose is attached. This galactosylceramide is further sulfated by cerebroside sulfotransferase (CST) [Handa et al., 1974; Honke et al., 1996; Hirahara et al., 2000], adding a sulfate group to the C3 position of the galactose moiety, to form sulfatide (Fig. 1). The same enzyme also produces sulfated lactosylceramide (Fig. 1) and seminolipid. Previous studies of sulfatide and its location to the islets of Langerhans [Buschard et al., 1993b, 1994], is performed by using the monoclonal antibody Sulph I [Fredman et al., 1988], which recognizes all three glycolipid antigens. Biochemical studies have supported that Sulph I staining of islets represents sulfatide, since sulfated lactosylceramide and seminolipid have not been found in adult human and rat pancreas. Sulfated lactosylceramide is a glycosphingolipid, which is mainly expressed in kidney and liver tissue [Ishizuka, 1997]. This molecule is comprised of a ceramide moiety, to which a glucose and a galactose group are attached to form lactosylceramide, after which a sulfate group is added (Fig. 1). Seminolipid is a glyceroglycolipid, predominantly expressed in testis [Ishizuka, 1997], containing a sulfated galactose group.

Previous studies [Hsu et al., 1998; Fredman et al., 2000] have revealed that the major sulfatide isoforms in pancreatic rat beta cells consist of saturated fatty acid chains of 16 or 24 carbon atoms, in contrast to sulfatide in the myelin, where the longer and hydroxylated carbon chains are more common. The fatty acid composition of glycosphingolipids is changing throughout development of the nervous system [Svennerholm and Stallberg-Stenhagen, 1968; Davison, 1971; Abe and Norton, 1974], and this phenomenon might be of importance for developmental processes [Rosenberg and Stern, 1966; Månsson et al., 1978]. Chain elongation, increased saturation, and hydroxylation of sulfatide occur when myelination begins. The

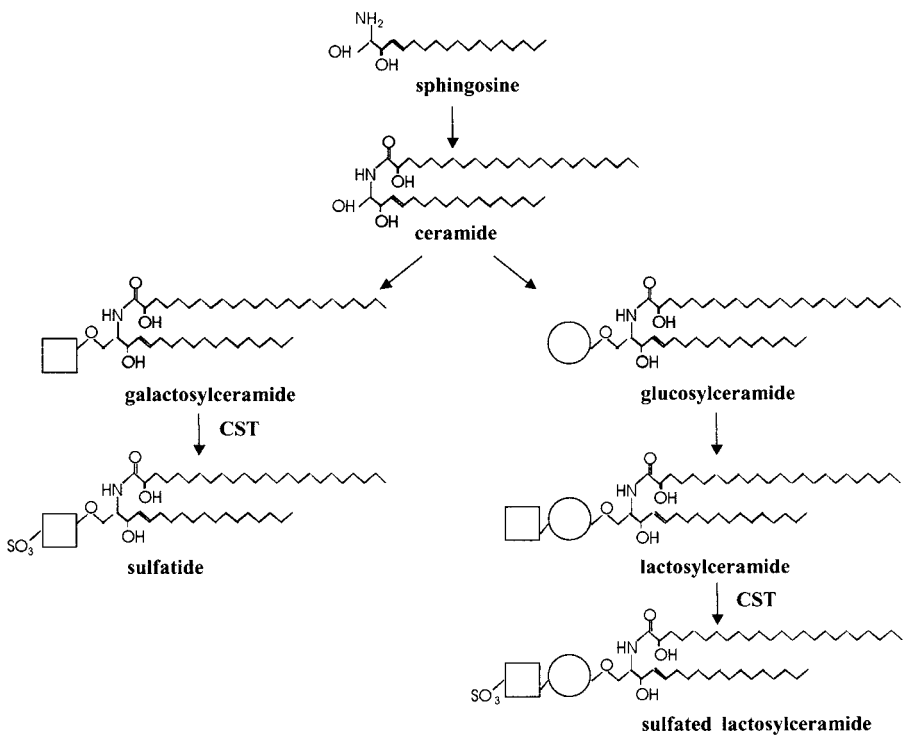

Fig. 1. Sulfatide and sulfated lactosylceramide synthesis pathways. Galactosylceramide and lactosylceramide are sulfated by the same cerebroside sulfotransferase (CST), present in the Golgi compartments, to form sulfatide and sulfated lactosylceramide, respectively. □, galactose, ○, glucose.

fatty acid composition of glycosphingolipids has been shown to be of importance for its biological function [Kannagi et al., 1983; Ladisch et al., 1989; Lingwood, 1996; Mamelak et al., 2001] and it has been implied that distinct glycosphingolipid isomers differ in their immunogenicity [Crook et al., 1986; Portoukalian, 2000].

Since type I diabetes is an autoimmune disease, it is relevant to explore the expression of proposed autoantigens during the fetal and neonatal period, when the distinction of self- and non-self antigens are going to be determined. In this study, we have investigated the expression of sulfatide and the closely related antigens, sulfated lactosylceramide and seminolipid in pancreas during development in human beings, rats, and mice. Moreover, we have studied differences in expression of these antigens, comparing the type I diabetes models, BB rat and NOD mouse, with the non-diabetic Lewis rat and BALB/c mouse, respectively. Differences in the fatty acid composition of sulfatide in the human pancreatic tissue and the different animal models were also examined.

MATERIALS AND METHODS

Human Tissue

Pancreas from 25 human foetuses, 16–24 weeks of age, were examined. The fetal tissues originated from California, and were collected in an ethically approved local project, which was approved by the Institutional Review Board, Cottage Hospital, Santa Barbara, CA 93105. Adult human pancreatic tissue was obtained from three kidney donors in the Scandinavian "Scantransplant" program. The use of tissue for scientific investigations has been approved by the Ethical Committee of the Danish Ministry of Health.

Animal Tissue

Pancreas from the type I diabetes rat model, the BB rat, and the control strain, non-diabetic Lewis rat, and the type I diabetes mouse model, NOD mouse, and the control strain, the non-diabetic BALB/c mouse were used in this study. Pancreas from 0- to 100-day-old animals (male) was examined. The number of animals investigated at each age varied between 2 and 10. Lewis and BB rats were purchased from Møllegaard (L1., Skensved, Denmark) and BALB/c and NOD mice from Bomholtgaard (Ry, Denmark) and bread in the stables at Bartholin Instituttet, Copenhagen, Denmark. The use of animals was approved by the local committee for animal studies. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Chemicals and Antibodies

Sulfatide used as standard was purified from pig brain (predominantly C24-C26 fatty acids with or without hydroxylation and C18:0) and sulfated lactosylceramide used as standard was prepared from post mortem kidney tissue of a patient with metachromatic leucodystorphy (MLD), according to the procedure previously described [Rosengren et al., 1989]. Seminolipid was a gift from Dr. Ineo Ishizuka, Department of Biochemistry, Teikyo University School of Medicine, Tokyo, Japan. The production and characterization of the monoclonal antibody Sulph I have been described previously [Fredman et al., 1988] and the antigens recognized are the glycolipids sulfatide, sulfated lactosylceramide, and seminolipid. The alkaline phosphatase conjugated goat anti-mouse IgG + IgM antibody was purchased from Jackson Laboratories (West Grove, PA).

Isolation of Rat and Mouse Pancreas

Animals were sacrificed (by carbon dioxide asphyxiation) and pancreas was isolated under sterile conditions and stored at -20°C until used for biochemical analysis.

Quantification of Sulph I Antigens

Extraction and separation of lipids, including sulfatide, sulfated lactosylceramide, and seminolipid, from pancreatic tissue was performed as described previously [Fredman et al., 2000]. Sulfatide, sulfated lactosylceramide, and seminolipid were identified and quantified by thin-layer chromatography-enzyme-linked immuno-sorbant assay (TLC-ELISA) [Davidsson et al., 1991; Pernber et al., 2002], using the monoclonal Sulph I antibody [Fredman et al., 1988]. Briefly, purified standards of sulfatide and sulfated lactosylceramide were applied as 5 mm lanes to TLC plates (10×20 plastic-backed high performance, Merck, Darmstadt, Germany) and chromatographed in chloroform/methanol/water (C/M/W, 65:25:4, by vol.). Seminolipid was quantified by using borate-impregnated TLC plates and chromatographed in C/M/W (75:25:3, by vol.) Plates were sequentially incubated with

the monoclonal Sulph I antibody, alkaline-phosphatase-conjugated anti-mouse antibody, and 5′-bromo-4′-chloro-3′-indolylphosphate. The procedure was performed at room temperature throughout. The intensity of the developed color was determined by densitometric scanning at 620 nm. The detection limit of sulfatide and sulfated lactosylceramide was <1 pmol/mg protein and seminolipid <10 pmol/mg protein.

Purification of Pancreatic Sulfatide Fraction for Fatty Acid Analysis by Electrospray Ionization-Mass Spectrometry (ESI-MS)

To obtain sulfatide in quantities sufficient for structural analyses with MS, it was necessary to pool material from various ages. The human fetal pancreatic sulfatide fractions from < 18 week- and >19 week-old-fetuses were pooled separately as were adult human pancreatic sulfatide fractions. Mice and rat pancreatic sulfatide fractions from 0- to 10-day-old animals and from animals at the age of > 60 days were pooled separately. The pooled samples were put on a Silica gel 60 (1 g, Merck) column and cholesterol was eluted with 8 ml chloroform and sulfatide with 15 ml C/M/W (65:25:4, by vol.). Saponification of the sulfatide fraction was performed as described previously [Fredman et al., 2000], after which the Silica gel 60 chromatography was repeated. The sulfatide fraction was desalinated on a Sephadex G-25 (Pharmacia & Upjohn, Uppsala, Sweden) column [Wells and Dittmer, 1963] and thereafter subjected to anion exchange chromatography on a 0.6 × 3 cm Fractogel® TSK DEAE-650 (M) column (Acetate form, Merck). Neutral glycosphingolipids were eluted with 5 ml C/M/W (60:30:4.5 by vol.) and sulfatide with 5 ml 0.02 M KAc in methanol. The latter fraction was evaporated, redissolved in 3 ml C/M (2:1 v/v) and 0.5 ml 0.9% NaCl, and re-partioned by adding 0.5 ml methanol and 0.5 ml 0.9% NaCl. This step was repeated and the sulfatide fraction again desalted on a Sephadex G-25 column, as described above.

The sulfatide fraction was chromatographed (C/M/W, 65:25:4 by vol.) as a 17 cm band on a TLC plate (10 × 20 plastic-backed high performance-TLC, Merck). The edges were cut off and further analyzed by the TLC-ELISA method described above. The gel fraction with migration similar to the visualized sulfatide was scraped off and mixed with 1–2 ml C/M/W (30:60:20 by vol.). Sulfatide was extracted from the gel by sonication, further purified on Sephadex G-25 columns as described above, and ceramide characterization was performed by ESI-MS.

ESI-MS. Samples were dissolved in C/M/W (30:60:20, by vol.) at a concentration of 10 μM before MS. Ceramide characterization was performed using ESI-MS in the negative mode on a quadrupol-time of flight mass spectrometer (Q-TOF, Micromass, Manchester, UK) equipped with a z-spray nanospray ion source. A capillary voltage of 900 V and a source block temperature of 80°C were used. Samples were manually loaded into nanoflow probe tips (type F thin wall, Micromass). For MS–MS, the gas cell was pressurized with argon gas and fragmentation of the precursor ion took place at a collision energy of 60 eV.

Statistics

Multiple linear regression analysis [Neter et al., 1996] was employed to study the effects of age and animal model on the concentrations of sulfatide and sulfated lactosylceramide. This was performed by incorporating dummy variables for animal model (group) and a multiplicative factor age × group to investigate a possible age dependent difference among the type I diabetes models and their normal counterparts. Differences between fetal and adult human sulfatide and sulfated lactosylceramide expression were investigated using Mann–Whitney's tests. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Human Pancreas

There was no correlation between increasing age and sulfatide concentration (pmol/mg protein) in fetal pancreatic tissue during week 16–22 of development (data not shown). Furthermore, no significant difference was observed between the sulfatide concentrations in the fetal tissue (800 ± 100 pmol/mg protein, n = 24) and those in adult pancreas (900 ± 100 pmol/mg protein, n = 3). During the same period of fetal development, sulfated lactosylceramide (concentration range of 100–300 pmol/mg protein) showed a significant decrease with increasing age (Fig. 2, $P = 0.04$). In contrast to sulfatide, as shown previously [Buschard et al., 1994], sulfated lactosylceramide could not be detected in human adult pancreatic tissue

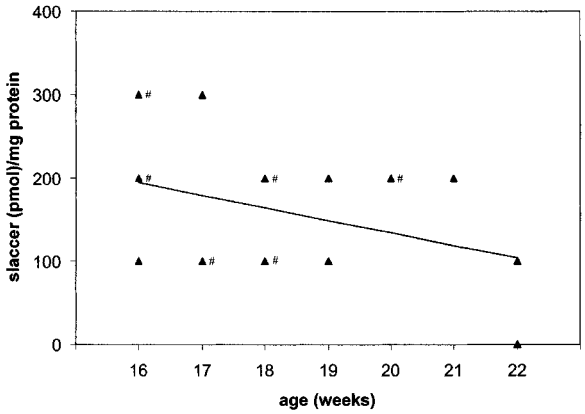

Fig. 2. Sulfated lactosylceramide (slaccer) expression in fetal pancreas during weeks 16–22 of development. Each individual value (pmol/mg protein) of sulfated lactosylceramide (▲) was plotted for the different ages (n = 1–9), and # indicates spots containing more than one value. The sulfated lactosylceramide concentration decreased significantly with increasing age ($P = 0.04$). The lipid isolation procedure as well as the (TLC-ELISA) method for quantification of sulfated lactosylceramide are described in Materials and Methods. The following regression model was used: $y = b_0 + b_1 \times \text{age}$.

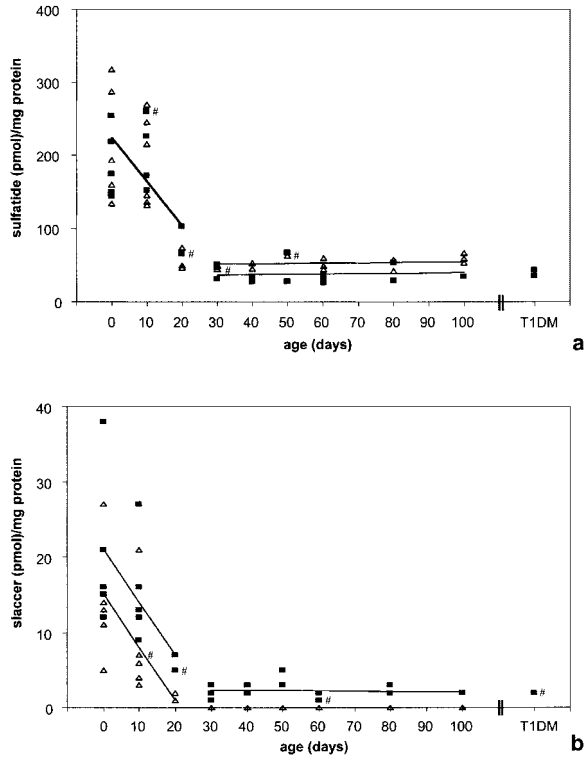

Fig. 3. Sulfatide (**a**) and sulfated lactosylceramide (slaccer, **b**) expression during development in Lewis and BB rat pancreas. Each individual value (pmol/mg protein) of sulfatide and sulfated lactosylceramide isolated from Lewis rat pancreas (△) and BB rat pancreas (■) were plotted for the different ages (n = 2–6), and # indicates spots containing more than one value. a: In both models, the sulfatide concentration was significantly decreased up to the age of 20 days ($P = 0.001$). Above the age of 20 days, Lewis rats showed significantly higher concentrations of sulfatide than the BB rats ($P = 0.0004$). b: In both models, the sulfated lactosylceramide concentration decreased significantly until the age of 20 days ($P = 0.0007$), while the BB rats had significantly higher expression ($P = 0.02$) than Lewis rats. After the age of 20 days, only BB rats expressed sulfated lactosylceramide. The lipid isolation procedure and the TLC-ELISA method for quantification of sulfatide and sulfated lactosylceramide are described in Materials and Methods. The following regression model was used: $y = b_0 + b_1 \times \text{age} + b_2 \times \text{group} + b_3 \times \text{age} \times \text{group}$. When b3 was non-significant, the age × group term was omitted from the model. T1DM = BB rats which had developed type I diabetes.

(<1 pmol/mg protein). Because of small fetal tissue samples, a round sum of each glycosphingolipid value was used. Seminolipid could not be detected in human fetal or adult pancreas.

Rat Pancreas

The sulfatide concentration in pancreas varied during development of non-diabetic Lewis rats in a similar manner as in the type I diabetes model, the BB rat, showing a significant decrease to the age of 20 days (Fig. 3a, $P = 0.001$). However, the Lewis rat showed a significantly higher concentration of sulfatide at ages above 20 days than the BB rat ($P = 0.0004$). In the BB rat, sulfated lactosylceramide was present in pancreas neonatally. In contrast to the Lewis rat, in which this glycosphingolipid disappears after 20 days of age (<1 pmol/mg protein, Fig. 3b), sulfated lactosylceramide in BB rat pancreas could be detected at all ages, although in minor amounts in the adults (Fig. 3b). This glycosphingolipid showed a significant decline in expression at age ≤20 days ($P = 0.0007$) in both rat models, where BB rats had statistically significant higher expression than Lewis rats ($P = 0.02$). The adult BB rats expressed similar amounts of the two antigens independent of whether or not they have developed type I diabetes (n = 2–3 in each age group above the age of 10 days and, n = 2 in the group which had developed type I diabetes). Seminolipid could not be detected in rat pancreas at any age.

Mouse Pancreas

The type I diabetes mice model, the NOD mouse, and the BALB/c mouse both showed a significant decrease in the sulfatide concentration (pmol/mg protein) with age during the first 20 days after birth (Fig. 4a, $P < 0.0001$). At ages above 20 days, there was an age dependent

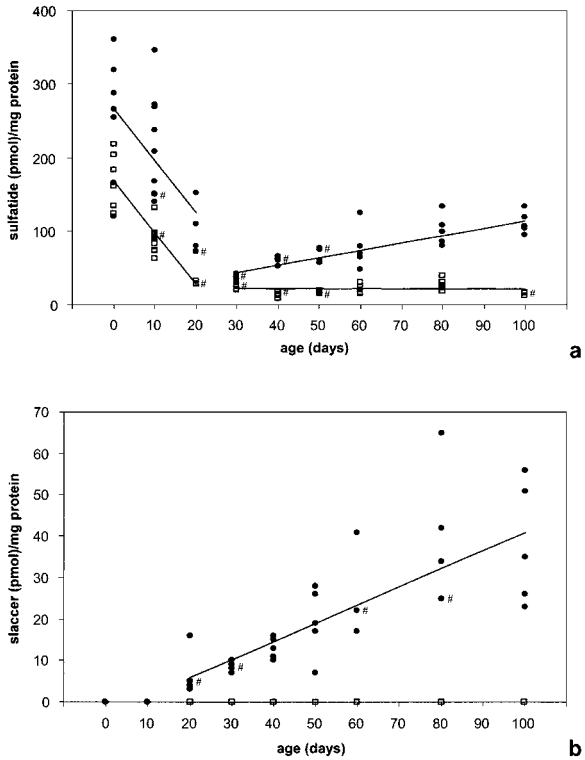

Fig. 4. Sulfatide (**a**) and sulfated lactosylceramide (slaccer, **b**) expression during development in BALB/c and NOD mouse pancreas. Each individual value (pmol/mg protein) of sulfatide and sulfated lactosylceramide isolated from BALB/c mouse pancreas (□) and NOD mouse pancreas (●) were plotted for the different ages (n = 5–10), and # indicates spots containing more than one value. a: In both models, the sulfatide concentration significantly decreased up to the age of 20 days ($P < 0.0001$). After the age of 20 days, an age dependent difference of sulfatide expression was found when comparing NOD and BALB/c mice ($P < 0.0001$). b: In NOD mouse, the sulfated lactosylceramide concentration increased gradually from day 20 ($P < 0.0001$). The lipid isolation procedure and the TLC-ELISA method for quantification of sulfatide and sulfated lactosylceramide are described in Materials and Methods. The following regression model was used: $y = b_0 + b_1 \times age + b_2 \times group + b_3 \times age \times group$. When b3 was non-significant, the age × group term was omitted from the model, and for sulfated lactosylceramide $y = b_0 + b_1 \times age$ was used.

difference of sulfatide expression when comparing NOD and BALB/c mice (Fig. 4a, $P < 0.0001$). The NOD mice showed a continuous increase with age while the BALB/c mice remained at a constant level of approximately 20 pmol/mg protein.

In contrast to the newborn BB and Lewis rats, NOD and BALB/c mice had no detectable levels of sulfated lactosylceramide in pancreas (<1 pmol/mg protein), either at the age of 0 days or at 10 days. Sulfated lactosylceramide was not detected in BALB/c mice at any age, while in the NOD mice, its expression gradually increased from day 20 (Fig. 4b, $P < 0.0001$). Seminolipid could not be detected in mouse pancreas at any age.

Fatty Acid Composition of Sulfatide in Pancreatic Tissue

The TLC-ELISA only shows tentative guidance of the sulfatide isomers present in the pancreas samples (Fig. 5). The upper band represents sulfatide containing long chain fatty acids and the lower band(s) represent short chain and hydroxylated fatty acids. To further explore the fatty acid composition of sulfatide, MS analysis was performed. The C24:0 and the C16:0 sulfatide isoforms, previously described as the major sulfatide isoforms in rat islets [Hsu et al., 1998; Fredman et al., 2000], was found in all of the pancreatic samples. There was also a hydroxylated form of the C16:0 sulfatide isomer present, which has not previously been detected in rat islets, in the two mice strains and the human fetal and adult pancreatic specimens. In the rat and human pancreatic tissues also sulfatide with C20, C22, and C23 fatty acid chains, both with and without hydroxylation could be detected. Owing to the low amounts of sulfatide after purification and difficulties in removing contaminating substances affecting

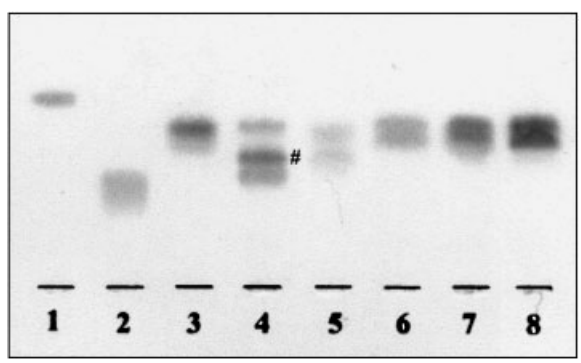

Fig. 5. Thin layer chromatography of Sulph I antigens in pancreatic tissue isolated from human, rat, and mouse. The lipid isolation procedure and the TLC-ELISA method are described in Materials and Methods. Lipids corresponding to approximately 1–5 mg tissue protein were applied. The amount of material applied to the TLC plate was not enough to visualize sulfated lactosylceramide in the BB rat. **Lane 1**: Seminolipid (120 pmol); **lane 2**, sulfated lactosylceramide (20 pmol); **lane 3**, sulfatide (40 pmol); **lane 4**, sulfatide/sulfated lactosylceramide fraction from NOD mouse; **lane 5**, BALB/c mouse; **lane 6**, BB-rat; **lane 7**, Lewis rat; **lane 8**, human pancreas. Rodents are 30–50 days of age. # indicate the lower sulfatide band in NOD and BALB/c mice, representing the hydroxylated form of C16:0 sulfatide.

the ionization of the sulfatide species, there was no way to obtain a reliable calculation of the proportions between the sulfatide isoforms detected.

In adult human pancreas, expression of C18:0 and C20:0 fatty acid isoforms of sulfatide appeared, in addition to those found in fetal tissue and, as mentioned above, in adult pancreas. There was no difference in the fatty acid chain of sulfatide between the human fetal pancreatic tissue of <18 weeks and >19 weeks. MS analyses of sulfatide isolated from rat or mouse pancreas revealed no differences in sulfatide isoform expression between neonatal and adult animals, either in the type I diabetes models or in their normal counterparts. The pancreatic isoform expression was not associated with the development of type I diabetes in the animal models.

DISCUSSION

This study shows that the potential diabetic autoantigen sulfatide [Buschard et al., 1993a] is expressed not only in adult pancreatic tissue, but also in human fetal and rodent neonatal pancreas, when the distinction of self- and non-self antigens are determined. Sulfatide has a different expression pattern in human than in rodents, concerning the amounts of sulfatide, and expression during development. The pancreatic expression of another sulfated glycosphingolipid, sulfated lactosylceramide, indicate that this molecule is a potential fetal/neonatal marker, at least in rat and human, which is further expressed in the type I diabetes animal models. These findings give further support to the possibility that sulfatide is a relevant autoantigen in type I diabetes, and in addition, that sulfated lactosylceramide might also function as a potential risk factor for disease development, at least in the animal models.

The difficulties in obtaining neonatal human pancreas and the fact that the human pancreatic autopsy material is affected by the rapid tissue destruction, makes it impossible to compare this state of human development to the corresponding period in rodents. Furthermore, fetal pancreas from rodents is difficult to dissect properly. Although samples from human and animal tissues were, for the reasons mentioned above, collected at different developmental stages, some comparisons can still be made. One obvious difference was that the sulfatide concentration in adult animals is about 20–50 times lower than in human adult pancreas. This is in agreement with immune electron microscopy studies, showing that human beta cell granules are more intensively stained with the Sulph I antibody (Buschard et al., unpublished communication) than mouse and rat granules [Buschard et al., 1993b]. Sulfatide expression in adult rat pancreas is, furthermore, about twice as high as sulfatide expression in pancreas of BALB/c mice. Correspondingly, increasing amounts of sulfatide is also observed in brain from lower vertebrates, moving to higher vertebrates [Vos et al., 1994; Ishizuka, 1997]. These observations might simply reflect the more primitive function(s) of lower vertebrates as compared with higher vertebrates.

Sulfated lactosylceramide is known primarily to occur in kidney and liver tissue [Ishizuka, 1997], but no reports are available about its occurrence in pancreas. In human and rat pancreas, this glycosphingolipid seems to be present during fetal life and neonatally, and then, in rats, disappearing before weaning. Why this antigen cannot be detected in neonatal pancreas from the mice models might have to do with detection limits, because the tissue material obtained from 0- to 10-day-old mice is much less compared to rat pancreatic tissues. There is also a possibility that sulfated lactosylceramide has a different developmental expression in mice than in rat and human pancreas. However, our results support the idea that sulfatide lactosylceramide might function as a fetal/neonatal marker in mammalian pancreas, which disappears or has reduced expression before weaning.

Interestingly, sulfated lactosylceramide is expressed during adulthood in the type I diabetes animal models BB rat and NOD mouse. The fact that the remaining beta cells of type I diabetics lose their first phase insulin response, which is a characteristic feature for immature beta cells, and the presence of the purposed fetal/neonatal marker sulfated lactosylceramide in pancreas of adult type I diabetes animal models might indicate that the beta cells decline to a "fetal mode of action." Although antibodies directed against sulfatide in type I diabetes patients do not react with sulfated lactosylceramide (Fredman et al., unpublished communication), these results does not rule out sulfated lactosylceramide as a potential risk

factor for type I diabetes, at least in the animal models. The interaction of sulfated lactosylceramide with the glycosphingolipid antigen presenting CD1 molecule(s) [Naidenko et al., 2000] has not been explored, but it is not unlikely that this molecule might be involved in the regulation of CD1d-restricted NKT-cells, which is thought to regulate an extremely diverse set of immunologic responses [Bendelac et al., 1997]. The alpha-galactosylceramide, which is an unnaturally occurring glycosphingolipid, is shown to be involved in the regulation of CD1d-restricted NKT-cells in NOD mice [Naumov et al., 2001] and that sulfated lactosylceramide, as an endogenous glycosphingolipid, might be involved in these processes is an interesting hypothesis which remains to be elucidated.

Previous studies on adult rats [Buschard et al., 1993b] and human pancreatic tissue (Buschard et al., unpublished communication) using electron microscopy, show Sulph I staining in the secretory granules of beta cells, representing intra-granule sulfatide. We cannot exclude the possibility that sulfated lactosylceramide is also located to these vesicles in fetal/neonatal and type I diabetes pancreatic beta cells, indicating that sulfated lactosylceramide might play a role, together with sulfatide, in the insulin processing during maturation of pancreas. The regulation of CST towards its substrates galactosylceramide, lactosylceramide and galactosyl-alkyl-acyl-glycerol is not known, but sulfated lactosylceramide is a low abundant glycosphingolipid in human adult tissues. The mechanisms underlying the regulation of the developmental expression of sulfated lactosylceramide in pancreas and its consequences remain to be elucidated.

Previous results have shown that treatment with sulfatide can delay the onset of type I diabetes in BB rats [Buschard et al., 1995] and prevent the disease in NOD mice [Buschard et al., 2001], further supporting the possible involvement of sulfatide in type I diabetes. However, the mechanism of involvement is unknown. The difference in amounts of sulfatide and sulfated lactosylceramide expressed in the two type I diabetes models in comparison with their controls indicates that if these glycosphingolipids are pathogenically involved in type I diabetes, they are probably involved at different stages of disease development in the two animal models. The BB rat shows insulitis prior to clinical diabetes, whereafter diabetes develops between 60 and 120 days of age [Mordes et al., 1987]. Thus, a similar sulfatide expression in pancreas of adult BB-rats compared to the BB-rats, which had developed diabetes was an expected finding.

The pancreatic sulfatide isoform expression seems not to be associated with development, as described previously, of glycosphingolipids in brain [Svennerholm and Stallberg-Stenhagen, 1968; Davison, 1971; Abe and Norton, 1974], or with the development of type I diabetes in the animal models. However, one interesting finding is observed from the MS analysis, i.e., the presence of a hydroxylated form of C16 sulfatide in mouse and human pancreatic tissue, which has not been found in rat tissue [Hsu et al., 1998; Fredman et al., 2000]. The most obvious changes in sulfatide expression during development was the findings that neonatal rodent pancreas shows a higher concentration of sulfatide than adult animals. The age related reduction before weaning correlates well with development of normal glucose response of rodent beta cells, but it is beyond the scoop of this study to further explore this phenomenon.

In conclusion, the proposed diabetogenic antigen sulfatide is present in pancreas during the critical period of self and non-self discrimination. In both animal and human pancreatic tissue, we show the presence of another sulfatide-related antigen, sulfated lactosylceramide, which might function as a fetal/neonatal pancreatic marker. Sulfated lactosylceramide is also expressed in adult tissue of the type I diabetes animal models, but its importance for immunological responses remains to be elucidated.

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